Autosomal-Recessive Mutations in MESD Cause Osteogenesis Imperfecta

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Abstract: Osteogenesis imperfecta (OI) comprises a genetically heterogeneous group of skeletal fragility diseases. Here, we report on five independent families with a progressively deforming type of OI, in whom we identified four homozygous truncation or frameshift mutations in MESD. Affected individuals had recurrent fractures and at least one had oligodontia. MESD encodes an endoplasmic reticulum (ER) chaperone protein for the canonical Wingless-related integration site (WNT) signaling receptors LRP5 and LRP6. Because complete absence of MESD causes embryonic lethality in mice, we hypothesized that the OI-associated mutations are hypomorphic alleles since these mutations occur downstream of the chaperone activity domain but upstream of ER-retention domain. This would be consistent with the clinical phenotypes of skeletal fragility and oligodontia in persons deficient for LRP5 and LRP6, respectively. When we expressed wild-type (WT) and mutant MESD in HEK293T cells, we detected WT MESD in cell lysate but not in conditioned medium, whereas the converse was true for mutant MESD. We observed that both WT and mutant MESD retained the ability to chaperone LRP5. Thus, OI-associated MESD mutations produce hypomorphic alleles whose failure to remain within the ER significantly reduces but does not completely eliminate LRP5 and LRP6 trafficking. Since these individuals have no eye abnormalities (which occur in individuals completely lacking LRP5) and have neither limb nor brain patterning defects (both of which occur in mice completely lacking LRP6), we infer that bone mass accrual and dental patterning are more sensitive to reduced canonical WNT signaling than are other developmental processes. Biologic agents that can increase LRP5 and LRP6-mediated WNT signaling could benefit individuals with MESD-associated OI.

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Autosomal-Recessive Mutations in *MESD* Cause Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) comprises a genetically heterogeneous group of skeletal fragility diseases. Here, we report on five independent families with a progressively deforming type of OI, in whom we identified four homozygous truncation or frameshift mutations in *MESD*. Affected individuals had recurrent fractures and at least one had oligodontia. *MESD* encodes an endoplasmic reticulum (ER) chaperone protein for the canonical Wingless-related integration site (WNT) signaling receptors LRP5 and LRP6. Because complete absence of MESD causes embryonic lethality in mice, we hypothesized that the OI-associated mutations are hypomorphic alleles since these mutations occur downstream of the chaperone activity domain but upstream of ER-retention domain. This would be consistent with the clinical phenotypes of skeletal fragility and oligodontia in persons deficient for LRP5 and LRP6, respectively. When we expressed wild-type (WT) and mutant *MESD* in HEK293T cells, we detected WT MESD in cell lysate but not in conditioned medium, whereas the converse was true for mutant MESD. We observed that both WT and mutant MESD retained the ability to chaperone LRP5. Thus, OI-associated *MESD* mutations produce hypomorphic alleles whose failure to remain within the ER significantly reduces but does not completely eliminate LRP5 and LRP6 trafficking. Since these individuals have no eye abnormalities (which occur in individuals completely lacking LRP5) and have neither limb nor brain patterning defects (both of which occur in mice completely lacking LRP6), we infer that bone mass accrual and dental patterning are more sensitive to reduced canonical WNT signaling than are other developmental processes. Biologic agents that can increase LRP5 and LRP6-mediated WNT signaling could benefit individuals with *MESD*-associated OI.

Osteogenesis imperfecta (OI) is a group of inherited disorders characterized by bone fragility. Clinical severity ranges from non-deforming to progressively deforming to neonatally lethal.[1] At present, mutations in 19 different genes have been associated with OI: *COL1A1* (MIM: 120150), *COL1A2* (MIM: 120160), *IFITM5* (MIM: 614757), *BMP1* (MIM: 112264), *CREB3L1* (MIM: 616215), *CRTAP* (MIM: 605497), *FKBP10* (MIM: 607063), *MBTPS2* (MIM: 300294), *P3H1* (MIM: 610339), *PLOD2* (MIM: 301865), *PPIB* (MIM: 123841), *SEC24D* (MIM: 607186), *SERPINF1* (MIM: 172860), *SERPINH1* (MIM: 600943), *SP7* (MIM: 606633), *SPARC* (MIM: 182120), *TENT5A* (MIM: 611357), *TMEM38B* (MIM: 611236), *WNT1* (MIM: 164820). ~85% of OI cases are caused by mutations in one of the two genes that encode type 1 collagen, *COL1A1* or *COL1A2*. Extra-skeletal manifestations seen in individuals with OI, such as blue sclerae, dentinogenesis imperfecta, and ligamentous laxity, depend upon the specific gene that is mutated and the nature of the mutation.

Here we report the discovery of an additional autosomal-recessive OI-associated gene (*MESD,* mesoderm

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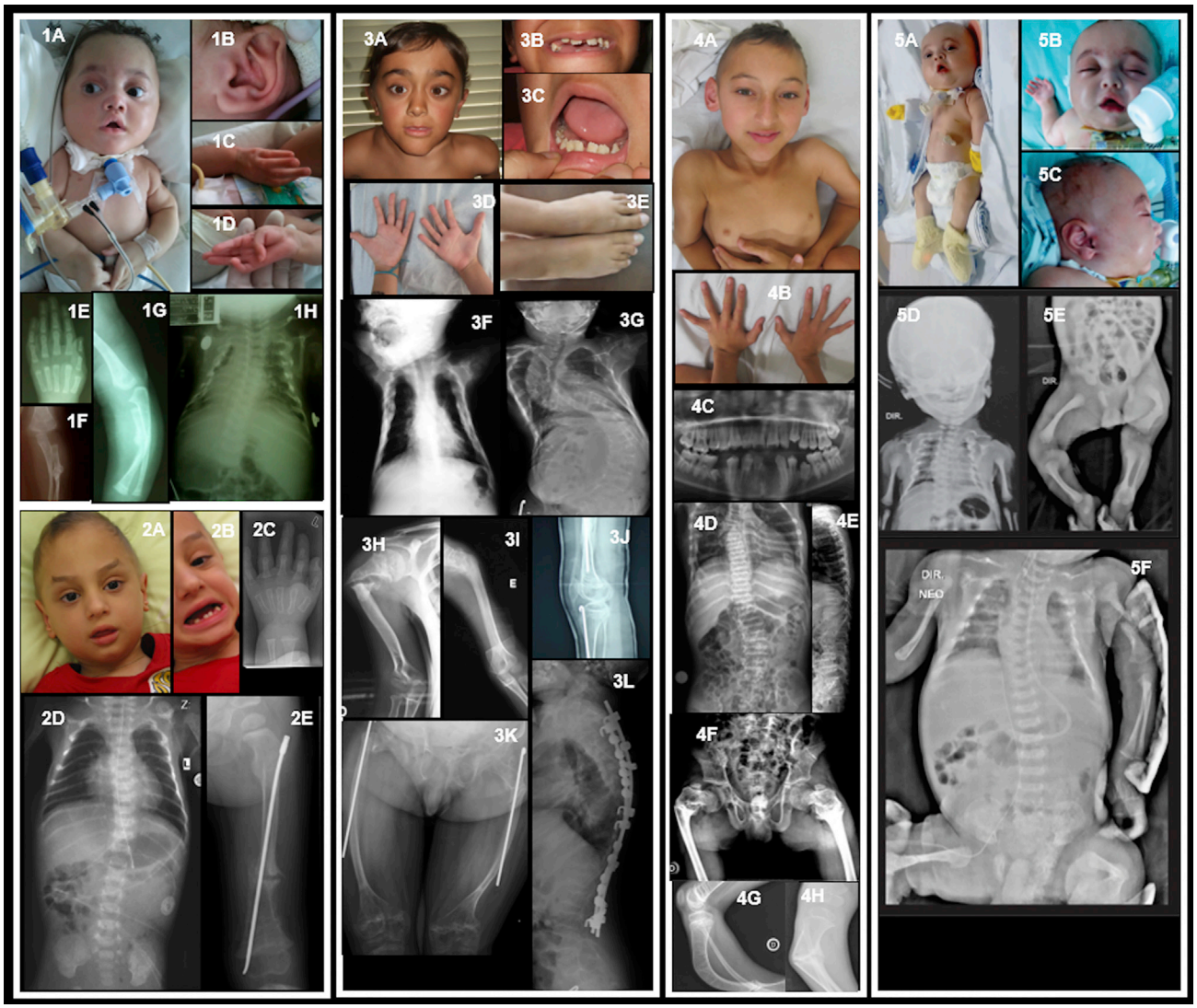

Figure 1. Clinical and Radiological Features of Individuals with *MESD* Mutations

(1) Individual 1 from Brazil (Family 1 IV-5)
(1A–1B) Facial dysmorphic features include bluish sclerae, mid-face hypoplasia, arched eyebrows, tented upper lip; also note tracheostomy, narrow chest, and rhizomelia; posteriorly rotated ears.
(1C–1D) Long fingers with adducted thumbs and contractures of the fifth digits.
(1E) Radiograph of left hand showing long, tapering fingers.
(1F–1G) Fracture of left ulna and radius at 5 months, healing with callus formation.
(1H) Radiograph of chest at 5 months showing bell-shaped, narrow chest with multiple (healing) rib fractures.
(2) Individual 2 from Turkey (Family 2 IV-1)
(2A–2B) Facial dysmorphic features include tall forehead, arched eyebrows with sparse lateral thirds, micrognathia, posteriorly rotated ears, widely spaced teeth, and oligodontia.
(2C) Radiograph of left hand at 8 months showing long, tapering fingers.
(2D) Chest radiograph at 5 months showing bell-shaped thorax, thin ribs with healing posterior rib fractures.
(2E) Fracture of the left femur at age 4 years, post-surgical rodding.
(3) Individual 3 from Portugal (Family 3 V-2)
(3A–3C) Facial dysmorphic features include triangular facies, arched eyebrows, left convergent strabismus, midface hypoplasia, thin upper lip, pointed chin, widely spaced teeth, and oligodontia.
(3D) Hands showing long fingers with mild contractures.
(3E) Feet showing 2-3 partial cutaneous syndactyly with overlapping toes.
(3F–3G) Chest radiographs at 6 months and 12 years, respectively, showing multiple rib fractures and progressive scoliosis with vertebral compression fractures.
(3H–3I) Upper limb radiographs showing right and left humeral fractures, respectively.
(3J) Right knee at age 8 years, showing signs of cyclical pamidronate therapy, rodding of the tibia, and very gracile fibula.
(3K) Aged 16 years, thin femurs with bilateral rodding of the femoral shafts post-fracture.
(3L) Lateral spinal radiographs at 12 years, showing osteopenia, progressive vertebral compression fractures, and spinal fixation.
(4) Individual 4 from Brazil (Family 4 II-2)

(legend continued on next page)

development gene, previously called *MESDC2* [MIM: 607783]); we describe clinical findings in five affected individuals from five independent, unrelated families; and we provide data that support the likelihood that OI-associated *MESD* mutations produce hypomorphic alleles rather than complete loss-of-function alleles. This study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee of the University of Cologne, Germany and the ethics committee of the University Medical Center Göttingen, Germany. All guardians signed informed consent for the molecular analysis, for publication of the results and corresponding clinical information, including clinical photographs, and for skin biopsies, where applicable.

Individuals who participated in this study had been clinically diagnosed with a progressive deforming form of OI (Figure 1); radiographic findings in all included osteopenia, skeletal deformity, healed fractures, and newly-acquired fractures. All affected individuals were offspring of consanguineous unions and had no prior family history of skeletal fragility. We performed whole-exome sequencing in order to simultaneously interrogate all known OI-associated genes. We used either the NimbleGen SeqCap EZ Human Exome Library kit (Roche NimbleGen) or the Agilent Sure Select Human All Exon V6 kit (Agilent Technologies) for exome capture. Library preparation, sequencing, and data analysis were performed as previously described.[2] Because of parental consanguinity, autosomal-recessive inheritance was considered likely, so for further study, we prioritized homozygous exonic and/or splicing variants whose reference population frequencies were <1% in gnomAD and ABraOM or <5% in in-house controls.

None of the affected individuals had likely pathogenic variants in any of the 19 genes which, when mutated, are known to cause OI. Instead, the affected individuals were each homozygous for a mutation in the third and final exon of *MESD* (GenBank: NM_015154.1), which is located downstream of the chaperone domain and upstream of the ER-retention domain (Table 1; Figure 2). These *MESD* variants were heterozygous in each parent and were not common polymorphisms in the Exome Variant Server, 1000 genomes, dbSNP, ExAC, or gnomAD databases. We found four different *MESD* mutations in the five affected individuals (c.607_611del [p.Thr203Alafs*26], c.631_632del [p.Lys211Glufs*19], c.632dupA [p.Lys212Glufs*19] found in two persons, and c.676C>T [p.Arg226*]). Notably, there are no high-quality loss-of-function variants annotated for *MESD* in the gnomAD database, while 7.9 such variants were calculated to be expected according to constraint metrics, indicating a high degree of loss-of-function intolerance of the gene (pLI = 0.91, observed/expected ratio = 0).

Salient clinical features in the individuals with *MESD* mutations are summarized in Table 1, and comprehensive clinical information is provided in the case reports in the Supplemental Data. Two individuals died from respiratory insufficiency before 18 months of age. The oldest affected individuals, when assessed at the ages of 10 years and 16 years, respectively, were non-ambulatory. Bluish sclerae were noted in the youngest individuals, but not in the older ones. None had dentinogenesis imperfecta, but the three older individuals had disorganized dentition and/or oligodontia (Figure 1). Global developmental delay or evidence of intellectual disability (ID) was present in three individuals (Table 1).

MESD mutations have not previously been associated with a human disease phenotype. In mice, homozygous loss-of-function *Mesd* mutations cause embryonic lethality during gastrulation.[3] Mouse embryos homozygous for deletions spanning *Mesd* can already be distinguished from WT embryos at embryonic day E7.5, when they show lack of mesoderm and failure to form a primitive streak.[3–5] These embryos do not survive beyond E11.5.[3] Functional studies of MESD and its *Drosophila* ortholog, Boca, indicate that these proteins are endoplasmic reticulum (ER) chaperones for canonical Wingless-related integration site (WNT) signaling receptors (LRP5 and LRP6 in mammals and Arrow in *Drosophila*).[5,6] In the absence of MESD, LRP5 and LRP6 cannot traffic to the cell surface.[5–9]

The likelihood that *MESD* mutations cause OI in these five individuals is supported by prior research demonstrating the importance of canonical WNT signaling in skeletal development.[2,10–18] Specifically, *LRP5* loss-of-function mutations are seen in the autosomal-recessive skeletal fragility disorder Osteoporosis-Pseudoglioma (OPPG) syndrome and in autosomal dominant juvenile osteoporosis.[10,11] Additionally, *WNT1* loss-of-function mutations have been found in autosomal-recessive OI and in dominant early onset osteoporosis.[2,12,13] Other data in support of the importance of canonical WNT signaling in skeletal development derive from human GWAS studies and from

(4A) He has minimal facial dysmorphic features, slightly upslanting palpebral fissures, and a pointed chin; sclerae are white; skeletal deformity with rhizomelic shortening is noted.
(4B) Impression of long fingers with mild interphalangeal contractures.
(4C) Panorex radiograph showing disorganized dentition, abnormal premolars, and missing lower jaw teeth (numbers 31, 32, and 41).
(4D–4E) Radiographs showing osteopenia, narrow chest, thin ribs with fractures, scoliosis, and vertebral compression fractures.
(4F–4H) Radiographs showing skeletal deformity and evidence of fractures of the femoral necks and tibiae.
(5) Individual 5 from Brazil (Family 5 IV-1)
(5A–5C) Facial dysmorphic features include round face, prominent eyes with long lashes and epicanthic folds, small nose with bulbous tip, tented upper lip, high and narrow palate, retrognathia, and posteriorly rotated ears; also note narrow thorax with inverted nipples, rhizomelic shortening of limbs, and long fingers with contractures.
(5D–5F) Radiographs showing generalized osteopenia, multiple fractures (clavicles, ribs, left humerus, femur), narrow thorax, and bowing of femurs bilaterally.

Table 1. Clinical Features of Individuals with Homozygous Mutations in *MESD*

Findings	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5
mutation in *MESD* (homozygous)	c.632dupA p.Lys212Glufs*19	c.631_632del p.Lys211Glufs*19	c.676C>T p.Arg226*	c.632dupA p.Lys212Glufs*19	c.607_611del p.Thr203Alafs*26
country of origin	Brazil	Turkey	Portugal	Brazil	Brazil
gender	female	male	male	male	male
consanguinity	yes	yes	yes	yes	yes
age at first visit (years)	after birth	3 years, 4 months	4 years, 6 months	10 years	6 months
age at last visit (years)	1 year, 5 months (death)	8 years, 4 months	11 years, 1 months	12 years, 2 months	7 months (death)
age at start of bisphosphonate treatment	15 days	3 years, 5 months	3 years, 6 months	5 years	not treated
age at end of bisphosphonate treatment	NA	NA	approx. 11 years	regular use since 2015 not treated from 2012–2014	not treated
birth weight and birth length	W = 2,135 g (−2.35 SD), L = 45 cm (−1.93 SD) (39 WG)	W = 1,550 g (−2.8 SD) L = 43 cm (−2.58 SD) (34 WG)	W = 3,730 g (+0.38 SD) L = 51 cm (+0.38 SD) (37 WG)	W = 2,730 g (−1.35 SD) L = 52 cm (+0.76 SD) (term)	W = 2,465 g (−1.73 SD) L = 42 cm (−2.94 SD) (37 WG)
confirmed prenatal fractures	yes	no	no	no	yes
age at first fracture (months)	prenatal	11 months	24 months	24 months	prenatal
color of sclera	bluish	white	white	white	bluish
eye abnormalities	NFA	no	no	no	no
dentinogenesis imperfecta	no	no	no	no	NA
disorganized dentition/clinical oligodontia	NA	yes	yes	yes	NA
radiographic evidence of oligodontia	NA	NA	NA	yes	NA
hypermobility of joints	no	no	NA	no	no
cardiac impairments	patent foramen ovale with a small left to right shunt	NA	NA	NA	no
hearing impairment	no	slight conductive hearing loss due to adenoid hyperplasia	no	NA	NA
old fractures of extremities	yes	yes (five fractures)	yes (right clavicle 2 years left femur 3 years 6 months right femur 4 years)	yes	yes (fractures of both humeri, ribs, femur, and clavicle)
vertebral/thoracic cage fractures	no	yes (multiple fractures of vertebrae, ribs, and both scapulae)	vertebral compression fractures	yes (vertebrae and ribs)	difficult to assess (multiple fractures of ribs).
retarded gross motor function	yes	yes	yes	yes	yes

L = length; NA = not available; NFA = not formally assessed; SD = standard deviation; WG = weeks of gestation; W = weight

studies in mice with germline and conditional knockout alleles of canonical WNT signaling pathway components. For example, loci identified by GWAS for bone mineral density and fracture risk include WNT pathway members.[14,15] In mice, bone-forming osteoblasts fail to differentiate when canonical WNT signaling is ablated.[16–18]

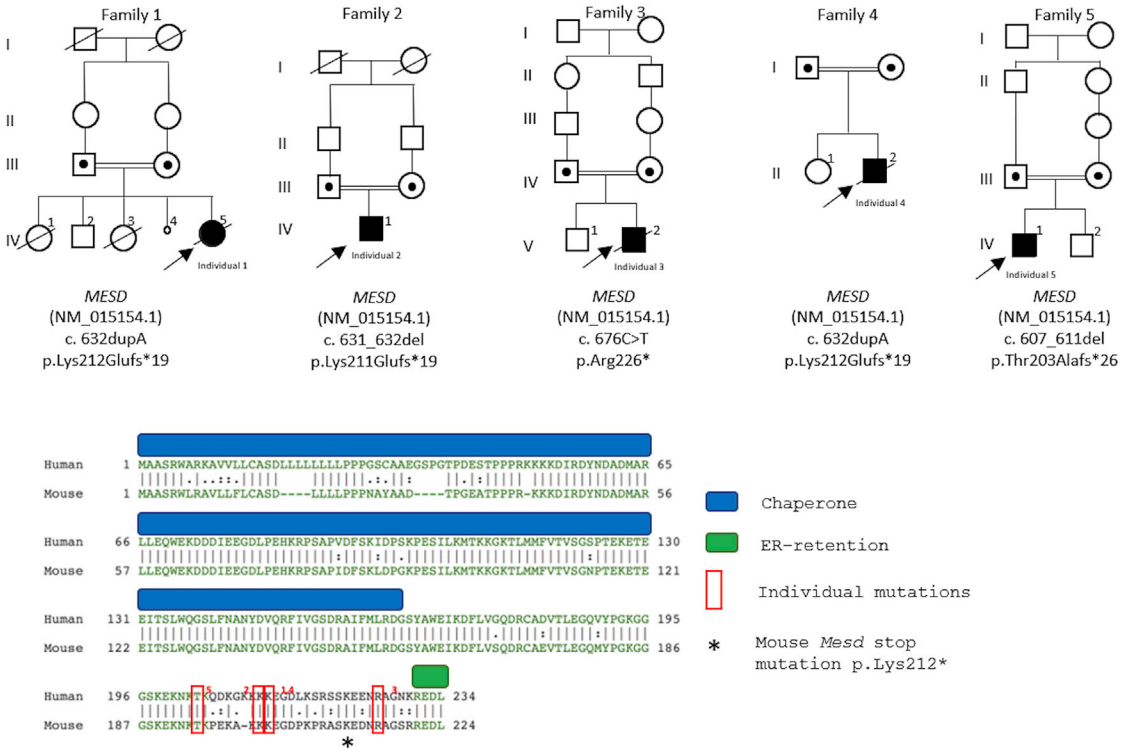

Figure 2. Pedigrees of the Five *MESD*-Associated OI Individuals, Their *MESD* Mutations, and the Location of these Mutations in the MESD Protein

Upper panel: Family pedigrees and mutations in *MESD* for each affected individual.
Lower panel: Alignment of human MESD with mouse MESD, showing important chaperone activity and ER-retention domains; the first amino acid residues affected by the four different affected individual mutations are noted with red rectangles and the amino acid residue converted to a termination codon in the mouse *Mesd* expression construct is asterisked.

Because embryonic lethality occurs in mice completely lacking MESD[3–5] or both LRP5 and LRP6,[19] we hypothesized that the *MESD* mutations responsible for human OI represent hypomorphic rather than amorphic alleles. Several lines of evidence support this speculation. First, OI-associated *MESD* mutations occur in the last exon, rather than throughout the gene, thereby making them likely to escape nonsense-mediated mRNA decay. Second, all mutations truncate or frameshift the protein after the 202nd amino acid residue, which is carboxy-terminal to the protein's chaperone activity domain.[20] Third, the OI-associated mutations all remove a highly conserved ER-retention domain;[5] the inability to retain or recycle MESD in the ER could reduce the protein's efficiency in serving as a chaperone.

To test this hypothesis, we studied fibroblasts from Individual 2 (Family 2 IV-1) and control fibroblasts, and we expressed wild-type (WT) and mutant *MESD* in HEK293T cells. We were able to RT-PCR amplify spliced *MESD* mRNA transcripts from WT-derived and Individual 2-derived fibroblast cultures. Thus, at least one *MESD* mutation does not cause nonsense-mediated mRNA decay (Figure 3A). Since the other mutations reported here are located in the same (and final) exon of *MESD*, likely the same will hold true for them. Also, an antibody whose epitope is near the amino-terminal domain of MESD and should be able to recognize WT and OI-associated mutant MESD only detected MESD in cell lysate from WT fibroblasts (Figure 3B); this is consistent with mutant MESD failing to remain intracellular when its ER-retention domain is lost. Because endogenous MESD levels can be rate limiting when LRP5 or LRP6 is overexpressed,[5] we performed transient transfection assays to determine whether mutant MESD retains an ability to chaperone LRP5. We used a previously described FLAG-epitope-tagged mouse *Mesd* expression construct that can chaperone human LRP5 in HEK293T cells.[21] We performed site-directed mutagenesis to modify this *Mesd* construct to create a truncation mutation (Quikchange Lightning Site-Directed Mutagenesis Kit, Agilent Technologies). The alteration p.Lys212* retains the chaperone activity domain and deletes the ER-retention domain, as do the OI-associated mutations (Figure 2 lower panel). Because lack of the ER-retention domain could allow mutant MESD to traffic outside the cell, we then examined the cell lysates and conditioned medium of transiently transfected HEK293T cells. In agreement with what we previously observed in control and Individual 2-derived fibroblasts, WT but not mutant MESD was detected in the cell lysate. In conditioned medium, only mutant and not WT MESD was detected (Figure 3C). These findings are consistent with the mutant MESD being synthesized but not retained within the ER.

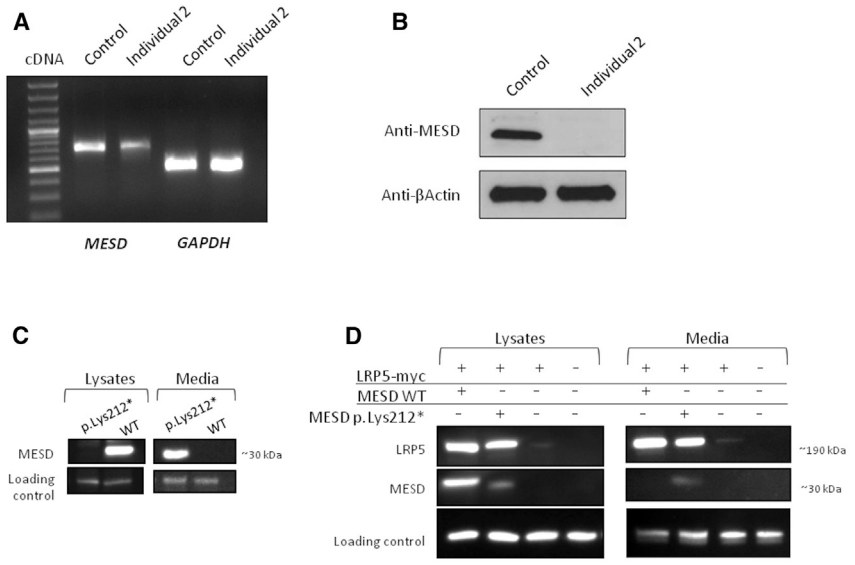

Figure 3. Individual 2-Derived and Transiently Transfected Cell Cultures Showing That OI-Associated MESD Protein Is Expressed and Capable of Serving as a Chaperone for LRP5, but Unable to Be Retained within Endoplasmic Reticulum of the Cell

(A) Agarose gel electrophoretic separation and fluorescence staining of RT-PCR amplimers spanning from exon 1 to exon 3 obtained from wild type (WT, control) and *MESD*-associated OI (Individual 2) cultured fibroblasts. Spliced *MESD* amplimers are detected in both samples; spliced *GAPDH* amplimers serve as a positive control.

(B) Immunodetection of MESD following SDS-PAGE in cell lysates from control and Individual 2 fibroblasts. A polyclonal antibody that detects an epitope near the amino-terminal domain of MESD should recognize both WT and mutant MESD. However, only WT MESD is present in the cell lysate.

(C) Representative immuno-blot following SDS-PAGE of FLAG epitope-tagged WT or mutant (p.Lys212*) MESD in cell lysate and conditioned medium from transiently transfected HEK293T cells. WT MESD is only detected in the cell lysate, whereas mutant MESD is only detected in the conditioned medium.

(D) Representative immuno-blots of the same membrane following SDS-PAGE using anti-myc antibody (for LRP5), anti-FLAG antibody (for MESD), anti-beta actin antibody (as a loading control for the cell lysate), and a non-specific cross-reacting band (as a loading control for the conditioned medium) of cell lysate and conditioned medium from HEK293T cells that were transiently transfected with expression vectors for myc-epitope tagged *LRP5* (*LRP5*-myc), FLAG-epitope tagged WT *MESD* (*MESD* WT), or FLAG-epitope tagged mutant *MESD* (*MESD* p.Lys212*). Note co-expression of *LRP5*-myc with either WT or mutant *MESD* increases the amount of immuno-detectable LRP5 in cell lysate and in conditioned medium, compared to when *LRP5*-myc is expressed alone. Also note that mutant MESD becomes detectable at low levels in cell lysate from HEK293T cells when transiently co-expressed with LRP5-myc. Mutant MESD is 13 amino acid residues shorter than WT MESD, which is why it migrates faster during SDS-PAGE.

We next determined whether the mutant protein could still chaperone LRP5. We co-expressed WT, or mutant, *MESD* with a Myc-epitope-tagged human *LRP5* that lacks a transmembrane domain.[21] Both WT and mutant MESD increased the abundance of epitope-tagged LRP5 in the conditioned medium (Figure 3D). Taken together, these data indicate that mutant MESD retains the ability to chaperone and traffic LRP5, though it may do so less efficiently than WT MESD because it fails to be retained in the ER.

The oligodontia, observed in Individual 4 for whom we have panorex films, may distinguish *MESD*-associated OI from other causes of OI. Reduced LRP6 signaling is the likely cause of oligodontia in this individual because oligodontia also occurs in persons who are heterozygous for *LRP6* loss-of-function mutations.[22] It is interesting that individuals with OI-associated *MESD* mutations do not have the eye defects seen in individuals who have the autosomal-recessive OPPG syndrome and who lack LRP5. Given that individuals with *MESD*-associated OI do not phenocopy humans or mice that are completely deficient for either LRP5 or LRP6, it seems likely that the skeletal fragility phenotype in those with *MESD* mutations is due to partial reductions in LRP5 and LRP6 signaling. This mechanism would be consistent with data from mice, in which partial reductions in LRP5 and LRP6 signaling affect bone mass without affecting other organ systems.[23] At present, we do not know the relationship between the *MESD* mutation and the ID we observed in several affected persons. These individuals had no variants in known ID genes. Interestingly, ID is also a variable clinical feature of *WNT1*-associated recessive OI.[24]

In the individuals described here, we attempted to increase bone strength and reduce deformity by treating with bisphosphonates, which have been useful in other types of OI. Unfortunately, we did not observe beneficial effects in the four individuals treated (see Case Reports in Supplemental Data). Bisphosphonates function primarily to reduce bone catabolism, rather than to promote bone anabolism. Since the skeletal fragility in these individuals is a likely consequence of low bone formation due to reduced WNT signaling, therapies that can enhance WNT signaling may have therapeutic efficacy. In this regard, it should be noted that Evenity (romozosumab-aqqg; Amgen) has recently gained FDA approval for increasing bone formation and bone mass in individuals with age-related osteoporosis. This therapeutic monoclonal antibody works by neutralizing sclerostin, an endogenous LRP5 and LRP6 inhibitor. Sclerostin neutralization in the LRP5 knockout mouse model of the OPPG syndrome produced significant increases in bone mass and bone strength.[25,26] This same response could occur in individuals with *MESD*-associated OI.

In conclusion, we identified *MESD* as an additional locus for autosomal-recessive OI. The likely mechanism of effect

for the four identified *MESD* truncating and frameshift mutations is the lack of ER retention, which reduces but does not eliminate the protein's role as a chaperone for the WNT receptors LRP5 and LRP6. However, since MESD when bound to LRP5 can block WNT signaling,[27] it is possible that the abnormal trafficking of mutant MESD outside of the cell causes a neomorphic effect. All five of the individuals with *MESD*-associated OI have a progressive deforming phenotype. Oligodontia may be a feature that separates this type of OI from the other known causes; however, too few individuals have been studied to reliably draw this conclusion. *MESD* joins a list of other WNT pathway components in which mutations can negatively affect bone mass accrual and bone strength. Therapies that enhance canonical WNT signaling in bone may significantly benefit these individuals.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2019.08.008.

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Declaration of Interests

The authors declare no competing interests.

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Web Resources

1000 Genomes Project, https://www.genome.gov
Arquivo Brasileiro Online de Mutações (ABraOM), abraom.ib.usp.br
Exome Aggregation Consortium (ExAC), exac.broadinstitute.org
Exome Variant Server, https://evs.gs.washington.edu
Genome Aggregation Database (gnomAD), https://gnomad.broadinstitute.org
OMIM, https://omim.org/
Single Nucleotide Polymorphism Database (dbSNP), https://www.ncbi.nlm.nih.gov

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